

# GPC5 Gene and Its Related Pathways in Lung Cancer

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Recently, a five-center collaborative study<sup>1</sup> reported that genetic variations of glypican-5 (*GPC5*) may significantly contribute to an increased risk of lung cancer in never smokers. *GPC5* gene expression levels in normal lung tissues were found significantly lower in individuals who carry high-risk alleles, and the *GPC5* expression level in adenocarcinoma tissue was significantly lower than in matched normal lung tissue. Reduction of expression of *GPC5* may lead to the development of lung cancer, suggesting that this gene normally functions as a tumor suppressor.

*GPC5* is a member of the glypican gene family. Glypicans are a family of heparan sulfate proteoglycans (HSPGs) that are linked to the exocytosomal surface of the plasma membrane by a glycosylphosphatidylinositol anchor. There are six glypican family members in the human genome (*GPC1* to *GPC6*).<sup>2</sup> *GPC1* is overexpressed in human pancreatic<sup>3</sup> and breast cancers.<sup>4</sup> *GPC3*, the family member that shows highest homology to *GPC5*, is overexpressed in neuroblastoma, Wilms' tumors,<sup>5</sup> and melanoma.<sup>6</sup> *GPC3* was also shown to be overexpressed in hepatocellular carcinoma,<sup>7</sup> and engineered *GPC3* overexpression in hepatocellular carcinoma cell lines was associated with modulated proliferation.<sup>8</sup> Missense mutations in *GPC3* are found in Simpson-Golabi-Beckel syndrome, which is associated with overgrowth and a reported predisposition to develop pediatric tumors.<sup>9</sup>

*GPC5* gene has eight exons encoding 572 amino acids and spans a large genomic region of 1.47 Mb at chromosome 13q31.3.<sup>10</sup> Alterations at the *GPC5* locus are a common event in various human tumors. Amplifications at 13q31-32 are frequently seen across several tumor types, including lymphomas, breast cancers, and neurologic tumors.<sup>11–13</sup> For lung cancer, an array comparative genomic hybridization (CGH)-

based study reported a homozygous deletion at 13q31.3 in a non-small cell lung cancer cell line.<sup>14</sup> Another array CGH-based study recently analyzed a series of 14 patients with 13q partial deletion syndrome and noted lung hypoplasia as one of the common phenotypes. Among the 14 patients, two had lung hypoplasia.<sup>15</sup> Amplification and overexpression of the miR-17-92 miRNA cluster at 13q31.3 was recently reported in lung cancers from one study<sup>16</sup>; another study showed that inhibition of miR-17-5p and miR-20a with antisense oligonucleotides can induce apoptosis selectively in lung cancer cells overexpressing miR-17-92.<sup>17</sup> When using TargetScan ([http://www.targetscan.org/vert\\_50/](http://www.targetscan.org/vert_50/)) to predict biologic targets of miRNAs, miR-17, miR-20a, miR-20b, miR-23a, and miR-23b were found to target *GPC5* 3' untranslated region. These results suggested that miRNA regulating *GPC5* expression may be important in the development of lung cancers and warrant in-depth investigation. Moreover, epigenetic silencing by hypermethylation of the CpG-rich region of *GPC5* leads to loss of *GPC5* function, which may in turn lead to carcinogenesis. Additional studies will be required to unravel the mechanisms of silencing or mutations of 13q31.3 region and its role in lung cancer.

Different single-nucleotide polymorphisms in *GPC5* have also been implicated in susceptibility to multiple sclerosis (MS).<sup>18</sup> A recent meta-analysis of cancer risk in MS has shown that the risk of lung cancer is significantly decreased in individuals with MS.<sup>19</sup> One study showed a particularly strongly decreased risk for cancers of the respiratory tract in MS patients.<sup>20</sup> HSPGs were found to be often expressed in reduced amounts in non-small cell lung carcinomas, particularly poorly differentiated tumors, compared with normal epithelia.<sup>21</sup> Our results also showed that *GPC5* expression is significantly lower in adenocarcinoma than in matched normal lung tissue.<sup>1</sup> In the ONCOMINE microarray databases, there are nine studies on lung cancer,<sup>22–30</sup> seven<sup>22–28</sup> of which included lung adenocarcinoma. Two datasets<sup>25,28</sup> showed significant downregulation of *GPC5* in adenocarcinoma tumors compared with normal lung tissue. Importantly, two studies<sup>25,26</sup> included smoking status information, and both showed lower expression in never smokers than in smokers. Four studies<sup>23,24,29,30</sup> reported the *GPC5* expression information from other histologic types, including carcinoid, squamous, small-cell carcinoma, and large-cell carcinoma, and showed no significant differences in *GPC5* expression between tumor and normal tissues. Thus, reduced *GPC5* expression could be specific to adenocarcinoma in never smokers. However, owing to sample sizes and the different characteristics of study samples available in ONCOMINE, this conclusion needs to be further validated. All the current

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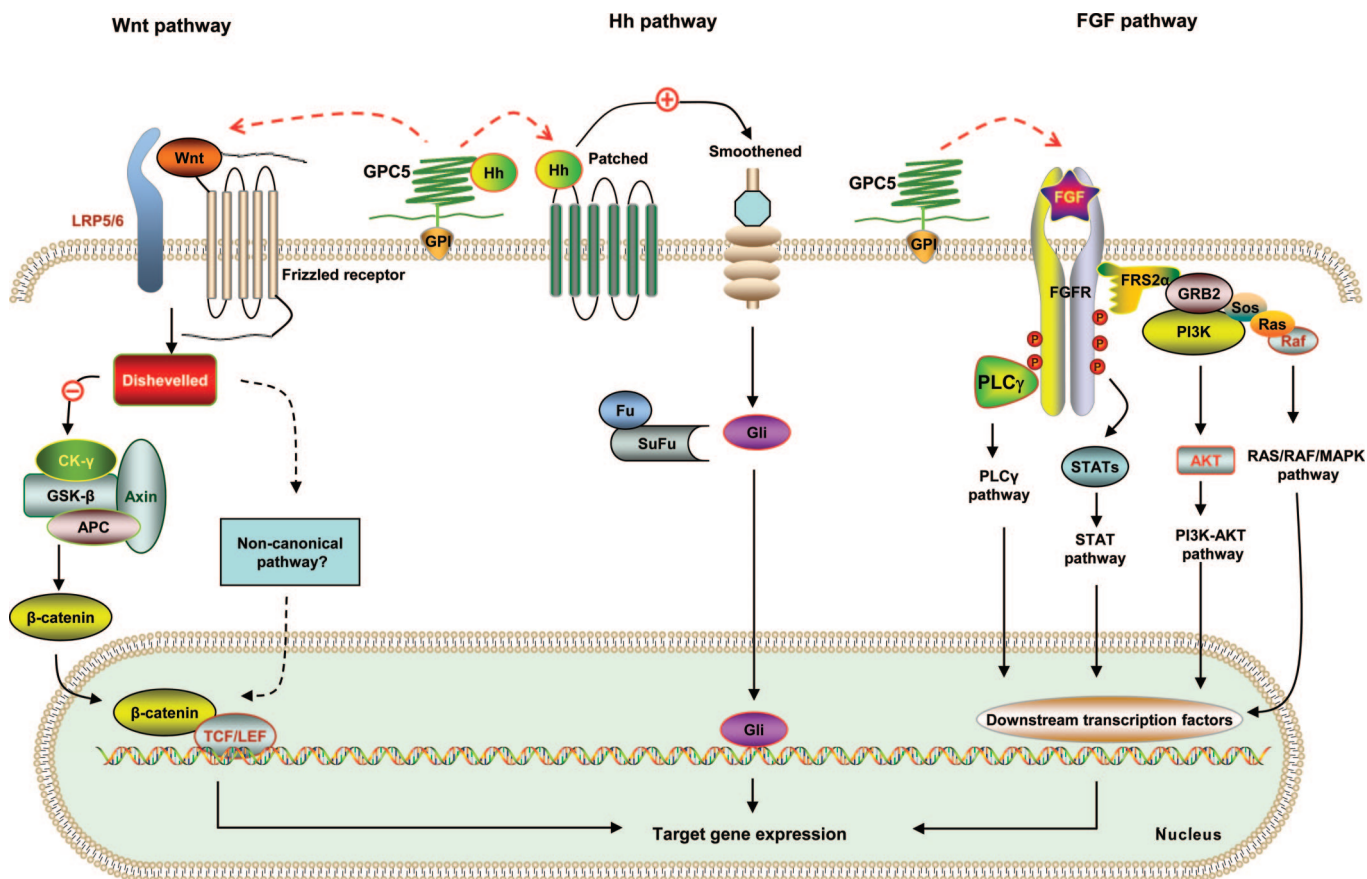
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evidence seems to support GPC5 to be a protective factor from developing lung cancer; mechanisms are open for further investigation.

Although there is no direct evidence for GPC5, HSPGs as a group are known to interact with many proteins including growth factors, chemokines, and structural proteins of the extracellular matrix to influence cell growth, differentiation, and the cellular response to the environment.<sup>31</sup> The main function of the membrane-attached glypicans is to regulate the signaling pathways of Wnt,

hedgehog (Hh), and fibroblast growth factors (FGF).<sup>32</sup> A recent study has shown that GPC5 increases proliferation in rhabdomyosarcoma through potentiating the effects of FGF2 and Wnt1; GPC5 enhanced the intracellular signaling of FGF2 and altered the cellular distribution of FGF2.<sup>33</sup> Previous and recent studies have highlighted potentially significant roles for Wnt, Hh, and FGF signaling pathways in lung cancer development.

Dysregulated Wnt signaling has been found in lung cancer, in particular, non-small cell lung cancer (NSCLC).<sup>34,35</sup>



**FIGURE 1.** Possible signal network of GPC5 in lung cancer development. The main function of membrane-attached GPC5 is thought to regulate the pathway signaling of Wnt, Hedgehogs (Hh), and fibroblast growth factors (FGFs). Depending on the biologic context, GPC5 can either stimulate or inhibit cell signaling activity. GPC5 may bind to Wnt and to the Frizzled receptor in association with the coreceptors LRP5/6. Activation of the Frizzled receptor results in subsequent activation of Dishevelled. Through an unknown mechanism, Dishevelled inhibits the function of a multiprotein complex including Axin, APC, CK- $\gamma$ , and GSK- $\beta$ , which blocks phosphorylation of  $\beta$ -catenin and prevents its degradation. Increasing levels of free  $\beta$ -catenin translocate to the nucleus, complex with the TCF/LEF cofactors, and activate target genes. In the case of the Hh pathway, it is proposed that GPC5 may inhibit the signaling by competing with Patched (the Hh receptor) for Hh binding. Binding Hh with the Patched receptor alters the interaction of Patched with Smoothened, a G protein-coupled receptor, resulting in the activation of Smoothened. This initiates a cascade of events resulting in the Gli entering the nucleus and acting as transcriptional activators. When the Hh signaling is lacking, Gli is bound to a multiprotein complex consisting of Fu and SuFu and will not enter the nucleus. GPC5 may activate the FGF pathway through unknown mechanisms. After FGF ligand binding and receptor dimerization, the kinase domains transphosphorylate each other, leading to the docking of adaptor proteins and the activation of four key downstream pathways: RAS-RAF-MAPK, PI3K-AKT, STAT, and PLC $\gamma$ . The biologic outcomes of FGF/FGFR activation are dependent on a complex network of signaling and transcriptional events regulated by multiple factors. CK- $\gamma$ , casein kinase  $\gamma$ ; GSK- $\beta$ , glycogen synthase kinase  $\beta$ ; APC, adenomatous polyposis coli; TCF, T cell-specific transcription factor; LEF, lymphoid enhancer-binding factor; Hh, hedgehog; Fu, fused; SuFu, suppressor of Fu; GPI, glycosylphosphatidylinositol; FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; FRS2 $\alpha$ , FGFR substrate 2 $\alpha$ ; GRB2, growth factor receptor-bound 2; Sos, son of sevenless; PLC $\gamma$ , phospholipase C  $\gamma$ ; STAT, signal-transducer and activator of transcription protein; MAPK, mitogen-activated kinase-like protein.

Several Wnt proteins are differentially expressed in NSCLC specimens, including Wnt1, -2, and -7a. Wnt1 and Wnt2 are overexpressed in NSCLC samples, and cancer cells expressing Wnt1 are resistant to apoptotic therapies.<sup>36,37</sup> Conversely, inhibition of Wnt1 and Wnt2 led to apoptosis in human cancer cells and reduced tumor growth in vivo and in vitro.<sup>36,38</sup> Wnt7a is decreased in NSCLC; its re-expression leads to growth inhibition of NSCLC cell lines.<sup>39</sup>

Persistent Hh pathway activation is seen in small cell lung cancer (SCLC), which is manifested by a high level of expression of sonic hedgehog (Shh), Patched, and Gli1.<sup>40</sup> Treatment of SCLC cell lines with a specific inhibitor of the Hh pathway (cyclopamine) produced tumor growth arrest. Cell lines were protected from cyclopamine inhibition by constitutive overexpression of the Hh pathway transcription factor Gli1.<sup>41</sup> Studies on cell lines demonstrated that five of seven SCLC cell lines expressed both Shh and Gli1 in contrast to NSCLC, which expressed only Shh but not Gli1. Analysis of clinical samples of human lung cancer tissue demonstrated 50% (5 of 10) of SCLC expressed both Shh and Gli1 compared with only 10% (4 of 40) of NSCLC.<sup>42</sup> Another study reported that 85% (34 of 40) of SCLC expressed Gli1 and more than 60% have a medium to strong expression correlating with increased Hh signaling.<sup>43</sup> Thus, it seems that the degree of dependence on Hh signaling varies among the subtypes of lung cancer.

Fibroblast growth factor (FGF) belongs to a family of ubiquitously expressed ligands, which bind to the extracellular domain of fibroblast growth factor receptors (FGFRs), initiating a signal transduction cascade, which promotes cell proliferation, motility, and angiogenesis. Dysregulation of the FGF signaling pathway has been associated with cancer development.<sup>44–46</sup> The FGF pathway has been shown to be activated in lung cancer.<sup>47–51</sup> Elevated levels of FGF and FGFR proteins have been detected in NSCLC cell lines and in human lung cancers.<sup>49,52,53</sup> Behrens et al.<sup>54</sup> described high levels of immunohistochemical expression of basic FGF, FGFR1, and FGFR2 in a large series of NSCLC specimens, including the two most frequent histologic types, squamous cell carcinoma and adenocarcinoma. Behrens et al. found higher levels of basic FGF and FGFRs expression in tumor cells than in adjacent normal bronchial epithelia at cytoplasmic localization in both squamous cell carcinoma and adenocarcinoma. In addition, in adenocarcinoma specimens, Behrens et al. detected differences in the expression of the three markers and patients' smoking status, with cytoplasmic FGFR1 expression being significantly higher in smokers and nuclear FGFR1 and FGFR2 significantly higher in never smokers. These differences highlight the potential differential role of these proteins in the pathogenesis of both smoking and nonsmoking-related lung cancers. Another recent study supports the previous studies and also provides molecular evidence for an active FGF autocrine signaling pathway in a subset of NSCLC cell lines.<sup>55</sup>

In summary, with all the information of GPC5 and the glypican family, we hypothesize that GPC5 regulates lung cancer development through a complex pathway network, particularly through Wnt, Hh, and FGF signaling pathways

and their interactions. As shown in Figure 1, depending on the context, GPC5 may have a stimulatory or inhibitory activity on these pathways, which are important in regulating cell proliferation, division, and survival. The challenge in the future will be to elucidate the precise regulation mechanisms of the GPC5 signaling pathway in lung tumorigenesis and in lung cancer of never smokers and smokers. A better understanding of GPC5's role in signaling pathways, particularly the upstream event that suppresses GPC5 expression and reduces functional GPC5, or the downstream event that unleashes the oncogenic processes may provide a unique opportunity for developing novel and effective strategies for early detection, targeted chemoprevention, and treatment of lung cancer.

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